

Avian Influenza: An Agricultural Perspective

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Recent outbreaks of infection with highly pathogenic H5N1 strains of avian influenza virus in poultry in Asia, Africa, Europe, and the Middle East have raised concern over the potential emergence of a pandemic strain that can easily infect humans and cause serious morbidity and mortality. To prevent and control a national outbreak, the US Department of Agriculture (USDA) conducts measures based on the ecology of avian influenza viruses. To prevent an outbreak in the United States, the USDA conducts surveillance of bird populations, restrictions on bird importation, educational outreach, and regulation of agricultural practices, in collaboration with local, state, and federal organizations. To manage an outbreak, the USDA has in place a well-established emergency management system for optimizing efforts. The USDA also collaborates with international organizations for disease prevention and control in other countries.

Avian influenza is a disease that mainly affects birds but can also occur in mammals. It is caused by influenza A virus, which is continuously evolving among its hosts, producing viral strains that have contributed to occasional pandemics in humans [1]. The potential impact of pandemic influenza was realized 3 times during the past century. The most devastating pandemic recorded, the 1918–1920 pandemic of “Spanish influenza,” is estimated to have caused the deaths of ~50 million people worldwide [2].

Concerns about another influenza pandemic have arisen as a result of recent outbreaks of infection with highly pathogenic strains of H5N1 influenza A viruses in poultry. These strains of H5N1 appear to be evolving rapidly, their host range has expanded to include migratory birds and mammals, and their geographic spread has increased from Asia to Africa, Europe, and the Middle East. To safeguard the United States from

avian influenza and to prevent another influenza pandemic, the US Department of Agriculture (USDA) conducts specific measures for preventing and for containing and eradicating outbreaks of influenza in animals, based on the ecology of avian influenza viruses.

THE ECOLOGY OF INFLUENZA A VIRUSES

The rapid rate of evolution of influenza A viruses is attributed to 2 properties of the virus genome: it comprises 8 segments of RNA, and viral RNA replication is susceptible to the infidelity of RNA polymerases, which lack proofreading functions [3]. Because the genome is segmented, it is particularly susceptible to reassortment. When 2 different influenza viruses infect a single cell, the 2 viral genomes may recombine to produce progeny viruses containing a mixture of RNA segments from the 2 parental viruses. These events result in a rate of evolution estimated to be 1 million times greater than that of eukaryotic genes [4] and allow for the acquisition of high pathogenicity and transmissibility to new species.

Although aquatic birds are the reservoir for all recognized influenza A hemagglutinin (HA) and neuraminidase (NA) subtypes, some viruses have been detected in other species. However, there appears to be a substantial degree of species specificity. For example, H1N1 and H3N2 have been detected in swine, whereas H3N8 and H7N7 are found in horses [1]. Recently, the

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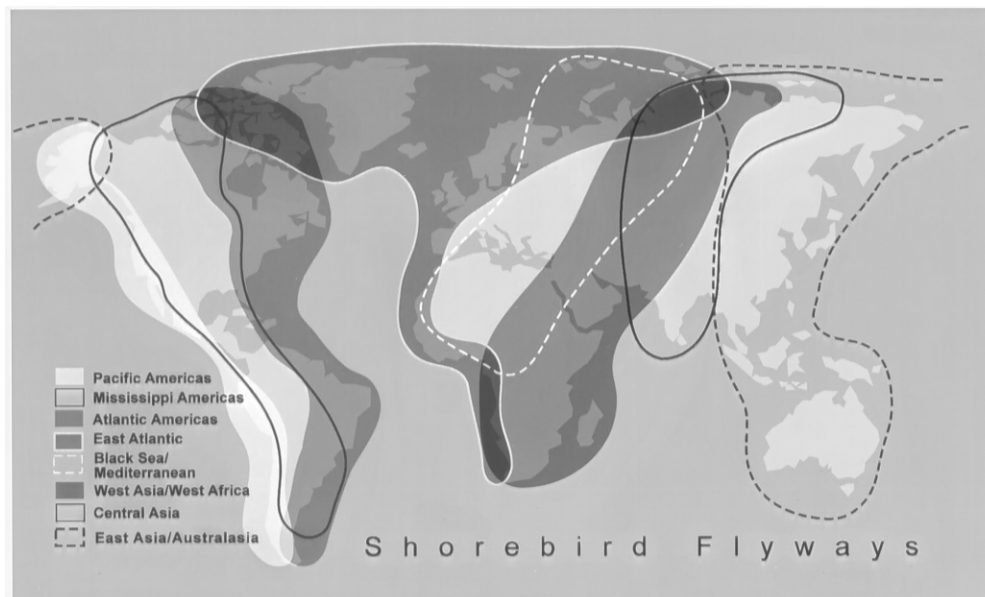


Figure 1. Global flyways, demonstrating partial overlap of flyways between regions [8]

horse H3N8 virus jumped the species barrier to cause infections and initiate an evolving outbreak in canines [5]. Furthermore, under experimental conditions, viruses derived from one species often do not replicate efficiently in another species. Inter-species transmission of influenza A viruses to a novel host can occur, resulting in extensive disease and an increased rate of viral mutation with viral adaptation [6]. Studies implicate multiple viral genes in partial host range restriction, although the precise roles of these genes in host specificity are unknown [1]. Attention has focused on HA, the major surface antigen of influenza, responsible for binding of virions to host cell receptors and for fusion between the envelope and the host cell. NA, another major surface glycoprotein of the virion, is essential in pathogenesis because it functions to free virus particles from host cell receptors, permitting progeny release from the cell and facilitating spread of the virus. The internal proteins encoded by influenza viruses may also contribute to host determination.

Aquatic wild birds are the natural reservoir of influenza A viruses and are thought to be the principal source of viral spread to other species. Influenza A viruses are ubiquitous in wild ducks and migratory shorebirds [1]. Aquatic birds can be infected with all subtypes of influenza A viruses, and infection is typically nonpathogenic, suggesting adaptation to the hosts. Compared with other species, aquatic birds appear to be in a state of evolutionary stasis with influenza A viruses.

Transmission of influenza A viruses from aquatic birds likely occurs through shared water sources. In aquatic birds, influenza A viruses replicate primarily in cells lining the gastrointestinal tract and are excreted in high concentrations in feces, which may contaminate water [1]. Influenza A viruses can remain

infectious in water for 100–200 days, depending on water temperature [7].

Transmission of influenza A viruses by infected migratory birds may result in wide geographic spread of viruses. Although flyways of bird populations are partially separated (figure 1), allowing distinct gene pools of viruses to develop [1], evidence suggests that there is interregional transmission by infected migratory birds. One study has demonstrated that H2 influenza A viruses isolated from shorebirds in North America between 1985 and 1998 contain genes belonging to a Eurasian lineage of H2 viruses [9]. Another study demonstrates that H2 viruses isolated in 2001 from migratory ducks that congregate in Japan on their flyway from Siberia contain genes derived from American and Eurasian lineages [10].

In terrestrial birds, most subtypes of influenza A viruses have been detected and are typically nonpathogenic, except for some strains of subtypes H5 and H7 [1]. Typical signs of infection with pathogenic strains include decreased egg production, respiratory symptoms, excessive lacrimation, sinusitis, cyanosis of unfeathered skin, edema of the head and face, ruffled feathers, diarrhea, and nervous disorders. Sources of infection may include infected aquatic birds and other animals, such as swine. Infected poultry may transmit viruses to each other and, possibly, to wild birds. During an outbreak of infection with a highly pathogenic strain of influenza, H7N7, in poultry in the Netherlands in 2003, H7N7 was detected in wild birds kept in captivity with infected poultry [11]. Although birds typically transmit influenza viruses via feces, there is evidence that quail shed influenza viruses primarily by the aerosol route [12]. Influenza A viruses have also been detected in the internal con-

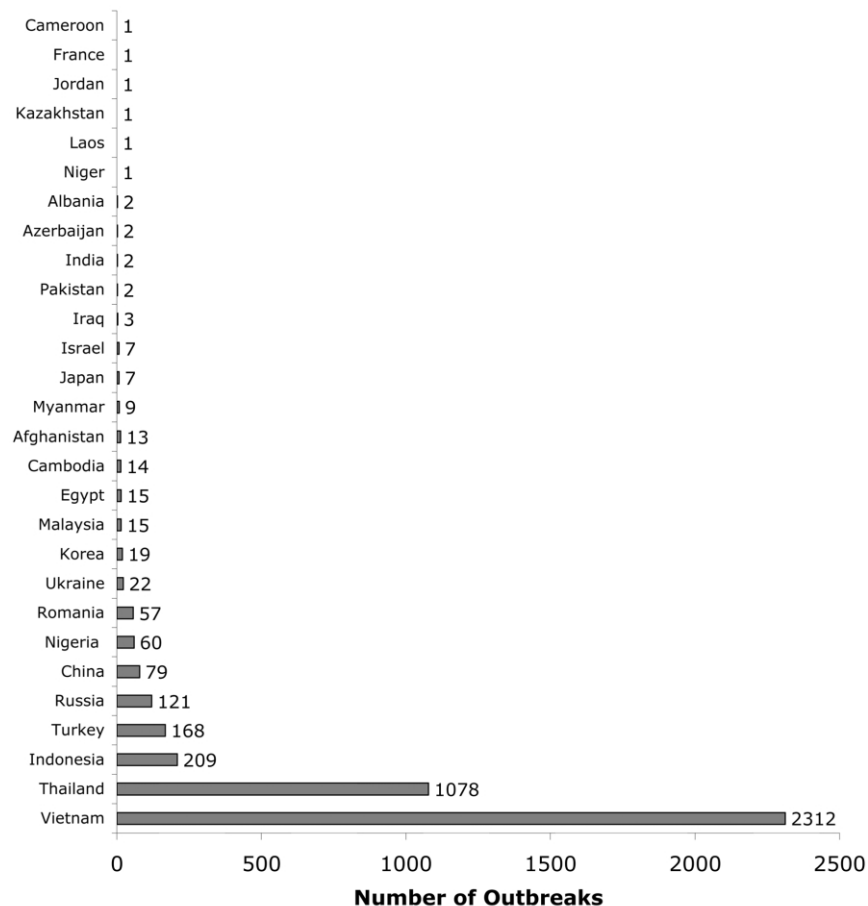


Figure 2. Number of outbreaks of infection with avian influenza virus subtype H5N1 in poultry, from the end of 2003 to March 2006 [21]

tents of eggs from infected chickens, another potential source of viral transmission [13]. Trade and illegal smuggling of poultry are, therefore, a means of disseminating the viruses.

Swine are a potential source of interspecies transmission of influenza A viruses [1]. In swine, as in humans, influenza A viruses are transmitted via the respiratory system and may cause nasal discharge, coughing, fever, labored breathing, conjunctivitis, and pneumonia [1]. Pigs may transmit infection to humans. Slaughterhouse workers frequently exhibit antibodies to swine influenza, and, occasionally, swine viruses are isolated from people with respiratory illness. There has been no proof in the recent past that transmission to humans has led to a human epidemic. Nevertheless, there is concern that pigs may serve as mixing species for reassortment between avian and human influenza viruses. Pigs express cell surface receptors for human and avian influenza viruses in the trachea, which provides a milieu conducive to coinfection and genetic reassortment between human and avian strains [14]. Pigs can be experimentally infected with human strains of influenza viruses [15]. Furthermore, during 1998, there were severe outbreaks of H3N2 infection in swine in the United States, and these

viruses were found to be double-reassortant viruses containing genes of human and swine viruses and triple-reassortant viruses containing genes of human, swine, and avian viruses [16, 17]. Reassortant viruses in pigs, containing human and swine gene segments, have also been detected in Japan and Europe [18, 19]. Triple-reassortant viruses, containing gene segments from avian, human, and swine strains, have been detected in pigs in Europe [20].

RECENT OUTBREAKS OF INFECTION WITH HIGHLY PATHOGENIC H5N1

Some noteworthy features of the ongoing outbreaks of infection with highly pathogenic H5N1 in Asia, Europe, Africa, and the Middle East are the number of affected animals and the widening geographic spread [21]. Since the initial outbreak in 1997 in poultry in farms and live-bird markets in Hong Kong, there have been >4000 outbreaks of H5N1 infection in poultry, mostly in Asia, and outbreaks in animals have been reported in at least 37 countries (figure 2). Although human activities may contribute to the geographic spread of H5N1, migratory

birds may also play an important role, as is suggested by outbreaks in migratory birds in remote areas such as Mongolia [22]. Outbreaks of H5N1 infection in some aquatic birds, including swans and certain types of geese, have been highly pathogenic, which is a new development, because influenza viruses have rarely been reported to be pathogenic in aquatic birds [23]. Another new characteristic of H5N1 viruses in aquatic birds is that viral shedding occurs from both the gut and respiratory tract. More-recent strains of H5N1 viruses have demonstrated low pathogenicity in experimentally inoculated ducks [24]. There has also been evidence of natural infection of apparently healthy migratory birds with H5N1 [25]. Because infection is not fatal, it is possible that these birds harbor the virus and transmit it interregionally during migration. Evidence of interregional transmission by migratory birds is provided by the isolation of genetically similar H5N1 viruses from migratory birds at 2 distinct sites, ~1700 km apart.

Another feature of these outbreaks is that there have been sporadic cases of direct transmission of viruses from poultry to humans, resulting in serious morbidity and mortality [26]. Most infected patients have had a history of direct contact with poultry: behaviors implicated in transmission include handling, plucking, and preparing diseased birds and consumption of ducks' blood or undercooked poultry. Although there have been suggestions of possible human-to-human transmission with close contact, there is no evidence of aerosol transmission. Specifically, seroprevalence studies of exposed health care workers and family contacts indicate that interpersonal transmission is inefficient.

H5N1 strains have infected and caused morbidity and mortality in felines, an unusual event [27]. This expanded range of hosts of H5N1 viruses increases the opportunity for viral evolution. During a December 2003 outbreak in poultry in Thailand, 2 tigers and 2 leopards at a local zoo were infected with H5N1 virus after being fed chicken carcasses, which resulted in high fever, respiratory distress, and death [28]. At the same zoo, there was also evidence of horizontal transmission of H5N1 between tigers: infection and disease spread to several tigers, which had not been fed raw chicken carcasses and were not in contact with other species [29]. The potential for intratracheal and horizontal transmission and transmission by feeding on virus-infected birds was confirmed experimentally in cats [30, 31].

Currently, there is no evidence that highly pathogenic strains of avian influenza, including H5N1, exist in the United States. Historically, there have been 3 outbreaks of infection with highly pathogenic strains in poultry in this country—in 1924, 1983, and 2004—but none of these outbreaks resulted in recognized human illness. In 1924, an outbreak of H7 infection was detected in and contained to live-bird markets in the eastern United States [32]. This outbreak was contained and eradicated. In 1983–1984, there was an outbreak of H5N2 infection in poultry in the northeastern United States [33]. This outbreak

was contained and eradicated after the destruction of ~17 million birds. In 2004, there was an outbreak of H5N2 infection in chickens in the southern United States. Although the clinical signs in the affected flock were mild and consistent with a low-pathogenicity strain of avian influenza, the amino acid sequences of the viral protein HA were consistent with those of highly pathogenic strains; therefore, the virus was classified as highly pathogenic [34]. The disease was quickly eradicated as a result of close collaboration between the USDA and state, local, and industry leaders.

EFFORTS BY THE USDA TO PREVENT AND CONTAIN OUTBREAKS OF INFECTION WITH HIGHLY PATHOGENIC STRAINS OF AVIAN INFLUENZA VIRUS

The USDA takes the following measures to prevent an outbreak of infection with highly pathogenic strains of avian influenza in the United States: surveillance of bird populations, restrictions on bird importation, educational outreach, and regulation of agricultural practices. Clearly defined policies are also established for containing and eradicating outbreaks of infection with highly pathogenic strains of avian influenza virus.

Surveillance is conducted in collaboration with federal, state, and industry partners to detect influenza A virus infection in live-bird markets, commercial flocks, backyard flocks, and migratory bird populations [32]. Although highly pathogenic strains of avian influenza virus are the primary target for surveillance, close attention is also given to 2 subtypes of low-pathogenicity strains, H5 and H7, which have the potential to mutate into highly pathogenic strains [35]. Diagnosis of infection is made using an assay developed by the Agricultural Research Service in 2002 [35]: a real-time, reverse-transcription–polymerase chain reaction test that produces results within 3 h and can detect H5 and H7 subtypes within a limit of $\sim 10^3$ – 10^4 gene copies [36]. This test was used successfully in the eradication of an outbreak in Texas in 2004 [35]. The test has been distributed to the National Animal Health Laboratory Network, which includes university and state veterinary diagnostic laboratories throughout the United States. Random testing occurs in live-bird markets, commercial flocks, wild migratory birds, and birds that show signs of illness [35].

To address the persistence of low-pathogenicity strains of avian influenza virus that are associated with the live-bird marketing system, random testing is performed at least quarterly in live-bird markets and in poultry distributors [37]. In addition, live-bird markets are required to undergo quarterly closure with depopulation, cleaning, disinfection, and down time of at least 24 h. This approach has had a significant impact on reducing transmission of influenza A viruses in live-bird markets [38]. Bird sources are also monitored: at least 30 birds per flock are tested 10 days before shipment to a distributor or

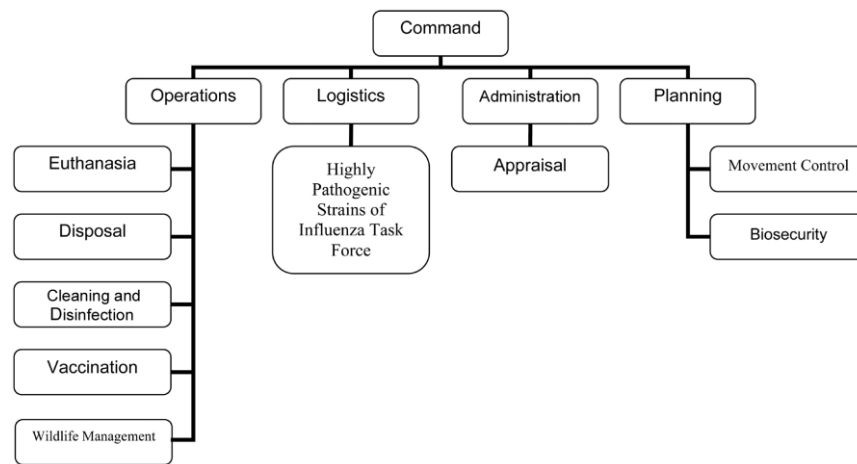


Figure 3. The Incident Command System structure in place at the United States Department of Agriculture (USDA) for responding to outbreaks of avian influenza [46]. The Incident Command System structure, a model for disaster-workforce organization, was developed by the USDA Forest Service and adopted by the Federal Emergency Management Agency and other emergency management organizations. The Incident Command System is all-inclusive and allows people from various state agencies, private industry, and multiple federal agencies to work together with a common goal and mission.

live-bird market [37]. If no cases are found, documentation is offered of the absence of infection with H5 and H7 subtypes of influenza A virus.

The National Poultry Improvement Plan provides a cooperative industry-state-federal program to certify that commercial poultry flocks are free of avian influenza and provides workshops for participants regarding diagnosis of avian influenza [39, 40]. To encourage noncommercial poultry and bird owners to report sick birds for testing, the Animal and Plant Health Inspection Service Veterinary Services division of the USDA conducts an outreach campaign called “Biosecurity for the Birds” [41].

A National Animal Health Surveillance System has also been created. This is a network of multiple government agencies and private entities, with the aim of protecting animal health, public health, national economic viability, and social welfare associated with animal populations [42]. As of October 2005, the National Animal Health Surveillance System has created an interagency H5N1 Working Group, under the direction of the Department of Homeland Security Policy Coordination Committee, with representation from the Department of Health and Human Services, the Department of the Interior, the International Association of Fish and Wildlife Agencies, the State of Alaska, and Animal and Plant Health Inspection Service Veterinary Services, to develop a surveillance plan to detect the first occurrence of H5N1 infection in wild waterfowl in Alaska [43].

The USDA maintains trade restrictions on the importation of poultry and poultry products from all affected countries. No birds can be imported from a country found to have the H5N1 strain of avian influenza. All imported live birds must be quarantined for 30 days at a USDA facility and tested for influenza

A virus before entering the United States. This requirement also covers returning pet birds of US origin [35]. To reduce the risk of avian influenza spreading to the United States, the USDA also collaborates with international organizations, including the World Health Organization for Animal Health and the United Nations Food and Agriculture Organization, to assist countries affected by highly pathogenic strains of avian influenza virus and neighboring countries with disease prevention, control, and eradication [44].

On detection of H5 or H7 subtypes of influenza A virus in a live-bird market, poultry distributor, or bird production site, the facility is required to undergo mandatory closure, depopulation, cleaning, and disinfection, and possible sources of infection are investigated. Before they are reopened, markets must be found to be negative on 3 consecutive monthly tests [37].

Indemnification programs are designed to encourage reporting of outbreaks and cooperation with disease control programs [45]. Federal indemnification is available for facilities that follow all program directives. Indemnification programs for outbreaks of infection with low-pathogenicity strains of avian influenza are generally managed by the states. Some industry associations have compensation funds. The USDA offers indemnification of 50% of fair market value for control of low-pathogenicity strains of avian influenza and 100% indemnification for outbreaks of highly pathogenic strains.

In the event of an outbreak of avian influenza, the USDA has a response structure in place called the “Incident Command System” (figure 3). This is a standardized organizational emergency management system designed to optimize efforts and minimize hindrance of efforts by jurisdictional boundaries [47]. This system organizes response efforts into 5 major manage-

ment categories: *command* sets objectives and priorities and organizes the response, *operations* conducts tactical operations, *planning* prepares and documents the plan, *logistics* provides the needed resources and services, and *finance/administration* monitors costs. The Incident Command System is the result of decades of lessons learned in the organization and management of emergency incidents, and it has been proven effective in several emergencies, including an outbreak of infection with low-pathogenicity strains of avian influenza virus in Virginia in 2002 [48].

On detection of highly pathogenic strains of avian influenza in poultry, the Animal and Plant Health Inspection Service would also quickly notify the Centers for Disease Control and Prevention to initiate their involvement, in coordination with state and local health departments, to minimize disease transmission to humans [44].

The Animal and Plant Health Inspection Service is currently considering the use of vaccines in poultry in the event of an outbreak in the United States [44]. As of November 2005, the USDA stockpiled 40 million doses of vaccine for 2 types of H5 and 2 types of H7 viruses [45]. Vaccination can act as a firewall against disease spread, reducing disease occurrence, the amount of virus in circulation, susceptibility to infection, and the level and duration of viral shedding. Vaccination may also induce immunity to more than one strain of a subtype and/or to more than one subtype of avian influenza virus [49–52]. Vaccination has been used successfully to control outbreaks of infection with low-pathogenicity strains of avian influenza in Italy in 2000–2002 [53] and Utah in 1995 [54], an outbreak of infection with a highly pathogenic strain in Mexico in 1995 [55], and an H5N1 infection outbreak in Hong Kong in 2002 [56]. Furthermore, in an experimental model, vaccination was found to reduce transmission to an extent that would prevent a major outbreak [57]. However, inappropriate use of vaccination may be dangerous, because it allows silent transmission of infection from asymptomatic birds [58], which may result in selection of antigenically divergent strains [59]. To avoid this potential danger, vaccination must go in tandem with monitoring systems to differentiate infected animals from vaccinated animals [60]. Another disadvantage of vaccination is that many countries will not import vaccinated poultry.

An approach that is not currently used by the USDA for controlling an outbreak of avian influenza is the use of antiviral medications in animals. This approach could pose a threat to the treatment of human infection with influenza A viruses [61]. Improper use of antiviral drugs may promote the development of drug-resistant strains, thus eliminating the possibility of using these drugs to treat human influenza cases. Already, a marker of amantadine resistance has been detected in isolates of H5N1 obtained from patients in China in 2003 and in isolates from birds and humans in Thailand, Vietnam, and Cambodia

[62]. A marker of oseltamivir resistance was detected in isolates from 2 of 8 Vietnamese patients [63].

The Agricultural Research Service is also conducting research to support efforts of the Animal and Plant Health Inspection Service, the Centers for Disease Control and Prevention, and the poultry industry to control avian influenza [64]. Researchers at the Agricultural Research Service are identifying and characterizing avian influenza in wild bird populations and domestic bird and swine populations, to better understand the basic ecology of avian influenza viruses [65]. They are studying factors that affect transmission between birds and molecular adaptation from migratory birds to poultry. Researchers are developing and evaluating techniques to predict which forms of low-pathogenicity strains of avian influenza might transform into highly pathogenic strains. They are also assisting in trade negotiations of poultry products by determining the risk of the presence of low- and high-pathogenicity viruses in poultry meat, the ability of pasteurization to inactivate influenza viruses in egg products, and the ability of cooking to kill highly pathogenic influenza viruses in poultry meat.

CONCLUSION

The expanding size and geographic spread of outbreaks of H5N1 infection throughout Asia and in Africa, Europe, and the Middle East has drawn attention to the potential threat of avian influenza to human health and the critical importance of containment and eradication in animals. It has also resulted in a greater appreciation and further examination of the ecology of influenza A viruses in animals. To safeguard the United States from highly pathogenic avian influenza, the USDA conducts measures to prevent, control, and eradicate outbreaks in animals. These measures include close collaboration with local, state, national, and international organizations and the implementation of a well-defined and well-established infrastructure for emergency response.

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